# BACTERIAL AND FUNGAL ORGANISMS IN THE VAGINA OF NORMAL COWS AND COWS WITH VAGINITIS

A Thesis

by

JAMES ROSS HUSTED

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

December 2003

Major Subject: Veterinary Microbiology

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# ABSTRACT

Bacterial and Fungal Organisms in the Vagina of Normal Cows and Cows with Vaginitis.

(December 2003)

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Previous studies on infections of the reproductive tract in various animal species have focused on uterine and cervical infections.[3,4,5,7,10,11] This study defined the bacterial flora from the vaginal fornix of 110 cows, with 55 exhibiting clinically healthy vaginas, and 55 demonstrating vaginitis. Aerobic, anaerobic, microaerophilic, and fungi cultures were done. In addition, cultures were performed for *Campylobacter* sp., *Ureaplasma* sp., *Mycoplasma* sp. and *Tritrichomonas foetus*. All 110 vaginal samples contained mixed aerobic bacterial cultures. The most frequent aerobic organisms isolated were *Acinetobacter lwoffii, Arcanobacterium pyogenes, Escherichia coli, Corynebacterium* spp., and *Streptococcus* spp. These organisms were isolated in both sampling groups, but more frequently from the vaginitis samples. Anaerobic isolates included *Peptostreptococcus* spp. and *Prevotella* spp. *Ureaplasma* sp., *Mycoplasma* sp., and *Tritrichomonas fetus* were not isolated in this study. There were not any true microaerophilic organisms isolated. Fungal isolates included *Aspergillus* sp., *Mucor* sp., and *Penicillium* sp. The clinical diagnosis of vaginitis was confirmed by histological results in 35 (64%) of the vaginitis samples.

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Also I need to thank Dwayne Anders for all of his help with collecting the samples from the slaughterhouse. Without his help the project would have taken twice as long to collect and culture the samples.

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# CHAPTER I

## INTRODUCTION AND LITERATURE REVIEW

Vaginitis, cervicitis, and bovine uterine infections are the causes of early death of the embryo, abortions, premature delivery, stillbirths, and death of newborn calves. A better understanding of the bacterial cause of vaginal and uterine infections in cows may provide clues for early treatment and prevention of these reproductive problems. Vaginitis, which may follow trauma or may be secondary to other infections, is inflammation of the mucous membrane of the vagina.[15] Vaginitis is most often caused by microorganisms such as *Arcanobacterium pyogenes*, *Escherichia coli*, *Streptococcus* sp., *Corynebacterium* sp., and *Haemophilus somnus*.[3,5,11,12,13,15]

It has been shown that there is a relationship between intra-uterine bacterial contamination and the development of endometritis in the postpartum cow with dystocia or a retained placenta.[5] In one study, the most frequent bacteria isolated were *Arcanobacterium pyogenes*, *E. coli*, *Streptococcus* sp., *Staphylococcus* sp., *Bacteroides* sp., *Fusobacterium necrophorum*, and *Clostridium* sp. Cows with retained placentas demonstrated a higher frequency of *E. coli* and *Clostridium* sp. than dystocia cows.[5] That study concluded that the development of endometritis later in dystocia and retained placentas in cows is partially mediated by early postpartum bacteria and endotoxins.

Another study examined obligate anaerobic and aerobic bacteria recovered from uteri of dairy cows with retained fetal membranes and postparturient endometritis.[3] Out of 77 dairy cows sampled, bacterial uterine infections were found in 55% of the samples.

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This thesis follows the style of the Journal of Veterinary Diagnostic Investigation.

*Arcanobacterium pyogenes* was found in 77% of the samples, suggesting that it is the predominant aerobic species involved in endometritis and pyometra.[3,5,11,13,15] In two of the samples a *Bacillus* sp. was found, and a single sample contained *Proteus* sp. These bacteria may or may not have been contaminants.

Another group took uterine samples for bacteriologic and histologic examination from 147 infertile cows during the late-luteal early-follicular stage of the estrous cycle.[14] This survey focused on bacterial cervicitis and metritis. The most frequent bacteria isolated included *Staphylococcus* sp., *Corynebacterium* sp., *Streptococcus* sp., *E. coli*, and *Arcanobacterium pyogenes*. Bacteria were isolated from 64% of samples. The most frequent histopathologic finding was mild, chronic endometritis in 76% of the samples.

A study was done to determine the frequency of *Ureaplasma diversum* in the vulvovaginal mucus of cattle with a history of reproductive disease.[1] Vulvar and vaginal infections are frequently associated with *U. diversum*. Fifty-nine of the 152 vulvovaginal mucus samples were positive for *U. diversum*. With regard to clinical symptoms, the data revealed a high association between *U. diversum* and purulent vaginal discharge due to a vaginal infection.

In a study done in 1986 *Acinetobacter calcoaceticus* (considered to be synonymous with *Acinetobacter lwoffii*) was isolated from cows with metritis.[4] *Acinetobacter calcoaceticus* has been isolated in pure culture from cows, which suggests that the bacterium could be associated with pathologic conditions of the uterus. The pathogenic potential of *Acinetobacter calcoaceticus* appears to be low, but it can be an opportunistic organism.

A report in 1984 describes the isolation of *Haemophilus somnus* from the vagina of a high proportion of dairy cattle with vaginitis and cervicitis.[12] In this particular study, a group of 40 heifers were sampled from a herd from in which 75% of the animals showed heavy mucopurulent vaginal discharge. *Haemophilus somnus* was isolated in 79% of the heifers, and isolated in pure culture from approximately 50% of the cases. In other studies *H. somnus* has been isolated from the vaginas of clinically normal cows. In one study, *H. somnus* was isolated up to 57% of the time from clinically normal vaginas.[12]

Purulent vaginal discharge became increasingly visible especially after mating in Australian dairy cattle.[14] A study investigated vaginal discharge in these particular cattle with a particular reference to *Haemophilus somnus*. Uterine swabs were taken from normal cows, cows with history of abnormal parturition, and sub-fertile cows. Media selective for *H. somnus* was used. The bacteria isolated most frequently included *Corynebacterium* sp., *Fusobacterium necrophorum*, *Peptostreptococcus indolicus*, *Bacteroides* sp., *Actinobacillus* sp., *Streptococcus bovis*, *E. coli* and *Haemophilus somnus*. *Haemophilus somnus* was the bacterial species isolated most frequently from samples taken from cows with inflammation or discharges. Three cases yielded *H. somnus* as a pure culture. The other bacterial species were evenly distributed between samples with infection and samples without infection. Half of the cultures from samples demonstrating metritis did not yield any bacterial growth.

According to official animal records, the culling of cows in Estonia during 1996 was primarily due to fertility problems.[10] An investigation looked into Estonian dairy

herds to study the postpartum period of cows with normal calving. The main emphasis of this study was placed on uterine infections. A total of 15 cows were studied from two different dairies. The cultures taken yielded a combination of bacteria including: *Bacteroides* sp., *E. coli*, *Streptococcus* sp., and *Staphylococcus* sp. as the most common isolates. The uterine flora contained mainly facultatively anaerobic bacteria, but in no case was *Arcanobacterium pyogenes* found. This is unusual because *A. pyogenes* is the most commonly isolated bacterium in almost all postpartum bacteriologic studies.[3,11,15]

A study done at the University of California Davis compared the bacterial flora of the normal canine vagina to that of canines with vaginal exudates.[8] Sixty-two clinically normal bitches were compared with 72 bitches with vaginitis. Both groups contained the same bacterial isolates with a few exceptions. The most common isolates included *Corynebacterium* sp., *E. coli*, *Moraxella* sp., *Arcanobacterium* sp., *Streptococcus* sp., and *Staphylococcus* sp. The only consistent difference between the two sources was the vaginal exudate group exhibited relatively higher numbers of bacteria than the normal canine vagina by microscopic examination.

A study was done to determine the bacterial flora of the vagina and uterus of healthy cats.[2] Aerobic bacteria were isolated from 52 of 53 vaginal swabs. The most frequent bacteria isolated were *Streptococcus* sp., *Corynebacterium* sp., *Arcanobacterium* sp., and *E. coli*. Comparing the normal flora to that of isolates found in vaginitis, the types of bacteria are frequently the same. As with the canine study, the main difference seen is that vaginal infections demonstrate an increased number of bacteria.

A more recent study was done to characterize the bacterial population of the genital tract of adult cats.[9] Specimens were collected from vaginal, uterine, or preputial mucosa from 66 female and 29 male cats. Aerobic bacteria such as *E. coli*, *Staphylococcus* sp., *Streptococcus* sp., and *Pasteurella multocida* were most commonly isolated from female cats. Anaerobic bacteria were only isolated in five percent of the female samples.

A literature review compared the bacterial flora in the vagina of healthy bitches to the bacterial flora of infertile bitches and bitches with vaginitis.[6] The authors findings suggested that the bacterial species isolated from bitches with reproductive disorders do not differ significantly from those found in healthy bitches. There is evidence that one or two bacterial species tend to dominate. *Mycoplasma* was recovered from vaginal samples of 30 to 88% of dogs without reproductive disorders. The anaerobic flora of the healthy vagina includes *Bacteroides* species as well as *Peptostreptococcus* species.

There is limited information on bovine bacterial vaginal flora. The majority of the articles published are studies based on uterine cultures. There has not been any research done that focuses directly on the identification of bacteria involved with bovine vaginitis. It is difficult to assign pathogenicity to one organism when a variety of species are isolated from one sample, because identification of a pathogen is complicated by the fact that the types of bacteria isolated are part of the normal flora. My study focused on the identification of bacteria that cause bovine vaginitis, as well as identification of the bacteria isolated from a clinically healthy bovine vagina.

# CHAPTER II

# MATERIALS AND METHODS

## Collection

The samples were collected from cattle at a commercial slaughterhouse plant located in San Antonio, Texas. Samples were taken on five different occasions during the summer and early fall of 2002. One hundred and ten samples were taken for this project. Fifty-five of the samples were from clinically healthy bovine vaginas, and 55 from cows with macroscopic vaginitis (vaginas with reddish mucosal surfaces or having a red or white discharge). Upon evisceration the reproductive tract was removed and the vagina was opened up to reveal the vaginal fornix. Each tract was swabbed twice circumferentially around the vaginal fornix, and then the swab (BBL Sparks, Maryland) was inserted into the external cervical os. Six swabs were collected from every sample and each swab was directly inserted into appropriate transport medium (BBL Sparks, Maryland). The swabs in the transport medium (BBL Sparks, Maryland) were taken to a room below the kill floor where the media was inoculated. No more than 20-minutes passed from the time samples were taken until the media was inoculated.

One swab was used to streak the blood and MacConkey agar plates (BBL Sparks, Maryland) for aerobic bacterial isolation. The swab was then placed in tryptose broth (Texas A&M University Veterinary Medical Teaching Hospital Media Kitchen). The next swab was used to streak blood agar plates (BBL Sparks, Maryland) for anaerobic bacterial isolation. This swab was placed in brain heart infusion broth with .1ml of oxyrase per 5ml of broth (Texas A&M University Veterinary Medical Teaching Hospital

Media Kitchen). Another swab was used to streak blood agar plates (BBL Sparks, Maryland) for microaerophilic bacteria. The same swab was used to streak out plates that contain selective media (Remel Lenexa, Kansas) for the isolation of *Campylobacter* sp. Another swab was used to streak and remain on mycobiotic agar slants (Remel Lenexa, Kansas) for fungal cultures. A swab was used to streak ureaplasma media plates (Texas A&M University Veterinary Medical Teaching Hospital, Clinical Microbiology Laboratory), and then placed in ureaplasma broth (Texas A&M University Veterinary Medical Teaching Hospital, Microbiology Laboratory). The last swab was used to streak mycoplasma media plates (Texas A&M University Veterinary Medical Teaching Hospital, Clinical Microbiology Laboratory), and placed in mycoplasma broth (Texas A&M University Veterinary Medical Teaching Hospital, Clinical Microbiology Laboratory). Scrapings of mucosa were taken directly from the fornix of the vagina to inoculate the InPouch TF pouch (Biomed diagnostics San Jose, California) for *Tritrichomonas foetus* identification. Finally, tissue samples taken from the vaginal fornix and the uterus were placed in formalin for histologic examination.

**Identification of Aerobic Bacteria**

The inoculated blood and MacConkey agar plates (BBL Sparks, Maryland) were incubated in air for 24-48 hours at 37°C. Plates were examined for growth, and pure sub-cultures were made from each isolate on fresh blood and MacConkey agar plates (BBL Sparks, Maryland). Isolates that did not grow were streaked onto fresh blood and MacConkey agar plates (BBL Sparks, Maryland) from the tryptose broth (Texas A&M University Veterinary Medical Teaching Hospital Media Kitchen) containing the original

swab. Once the Gram reaction was determined, bacteria were identified using the Vitek Microbial Identification System (bioMerioux Hazelwood, Missouri).

Gram-negative isolates were identified by using the Gram-negative Identification card in the Vitek system (bioMerioux Hazelwood, Missouri). Gram-negative bacteria that were not identified by the Vitek Microbial Identification System were identified by the API 20E strip (bioMerieux Hazelwood, Missouri). Gram-positive isolates were identified using the Gram-positive card in the Vitek system (bioMerieux Hazelwood, Missouri). *Corynebacterium* spp. were identified using the API CORNYE strips (bioMerieux Hazelwood, Missouri). *Streptococcus* spp. that were not identified by the Vitek Microbial Identification System were identified by using the API STREP strips (bioMerieux Hazelwood, Missouri)

**Identification of Anaerobic Bacteria**

Inoculated blood agar plates (BBL Sparks, Maryland) were incubated for 36-48 hours at 37°C in an anaerobic environment using AnaeroGen packs (Oxoid Basingstoke, Hampshire, England). Pure cultures were made from each isolate, and an aerotolerance test was performed on each isolate to distinguish obligate anaerobes from facultative aerobic bacteria. Samples that did not grow were restreaked from brain heart infusion broth with .1ml of oxyrase per 5 ml of broth (Texas A&M University Veterinary Medical Teaching Hospital Media Kitchen) containing the original swab, and incubated for 36-48 hours at 37°C in an anaerobic enviroment. Strictly anaerobic bacteria were Gram-stained and identified using the Vitek Microbial Identification System (bioMerioux Hazelwood, Missouri).

**Identification of Microaerophilic Bacteria**

After an incubation period of 36-48 hours at 37°C, blood agar plates (BBL Sparks, Maryland) were removed from the jar providing 10% carbon dioxide environment by the use of a CampyGen pack (Oxoid Basingstoke, Hampshire, England). Pure cultures were made from each isolate, and an aerotolerance test was performed on each isolate to distinguish true microaerophilic from facultative aerobic bacterial isolates. True microaerophilic bacteria were identified according to Gram reaction, and by using biochemical tests.

**Identification of *Campylobacter* sp.**

Campylobacter selective plates (Remel Lenexa, Kansas) were incubated for 36-48 hours at 37°C in a 10% carbon dioxide environment using a CampyGen pack (Oxoid Basingstoke, Hampshire). An aerotolerance test was performed on all bacterial isolates that grew on the media. Isolates that demonstrated colony morphology similar to *Campylobacter* sp. were gram stained (gram negative curved rods) and identified accordingly.

**Aerotolerance Test**

Each bacterial isolate that was suspected of being a strict anaerobe or microaerophilic bacterium was streaked onto a fresh blood agar plate (BBL Sparks, Maryland). The inoculated plates were incubated aerobically at 37°C for 24 to 36 hours. After incubation, plates were examined for growth of bacteria. Bacterial isolates that grew were considered to be facultative bacteria. Bacterial isolates that did not grow

aerobically were considered strict anaerobes or microaerophilic bacteria depending on their method of culture.

**Identification of *Ureaplasma* Bacteria**

The inoculated media and broth selective for *Ureaplasma* (Texas A&M University Veterinary Medical Teaching Hospital, Clinical Microbiology Laboratory) was incubated at 37°C in a 10% $CO_2$ environment using a CampyGen pack (Oxoid Basingstoke, Hampshire, England). Plates were examined for growth, and identified by colony morphology, as well as broth color change. Further identification may be pursued using polymerase chain reaction (PCR).

**Identification of *Mycoplasma* Bacteria**

*Mycoplasma* bacteria were identified by colony morphology. Media and broth selective for *Mycoplasma* (Texas A&M University Veterinary Medical Teaching Hospital, Clinical Microbiology Laboratory) were incubated at 37°C in a 10% $CO_2$ environment using a CampyGen pack (Oxoid Basingstoke, Hampshire, England).

**Identification of Fungi**

Fungal isolates were incubated at room temperature for seven to ten days on mycobiotic agar (Remel Lenexa, Kansas). Sabourads Dextrose agar was used on the first ten isolates (BBL Sparks, Maryland), and incubated in the same method. Isolates were fixed on slides and stained with Lactophenol Cotton Blue Stain, and identified by examination under a light microscope. Identification was done using Medically Important Fungi a Guide to Identification 3rd Edition as a reference. Fungal isolates were identified to the genus level.

**Identification of *Tritrichomonas foetus***

The InPouch TF pouch (Biomed diagnostics San Jose, California) was incubated at 37°C. The pouches were examined microscopically for the presence of *Tritrichomonas foetus* at 24, 48, and 72 hours after inoculation. The InPouch TF pouch was examined directly by light microscopy at a magnification of 10X. Pouches that did not display *Tritrichomonas foetus* movement after the 72-hour incubation period were considered negative.

**Histopathologic Examination**

Cross sections of the vaginal fornix and the uterus were fixed in 10% buffered formalin were paraffin embedded, and stained for routine light microscopy.

**Statistical Analysis**

The data collected from this study was analyzed by using the odds ratio. Each bacterial and fungal isolate occurrence was compared with vaginitis and non-vaginitis samples. A ratio above one indicates a relationship between exposure and disease.

# CHAPTER III

# RESULTS

## Aerobic Bacteria Isolation Results

Aerobic bacteria were isolated in all of the 110 samples. The most frequent aerobic bacteria isolated include *Acinetobacter lwoffii, Arcanobacterium pyogenes, Escherichia coli, Corynebacterium* sp., *Streptococcus* sp., and *Staphylococcus* sp. With a few exceptions, the aerobic bacteria isolated were relatively the same for both vaginitis and non-vaginitis samples. The major bacteriological difference being vaginitis samples have more isolates than non-vaginitis. A comparison of the number of each aerobic bacteria isolated in vaginitis and non-vaginitis samples is shown in Table 1. *Arcanobacterium pyogenes* was isolated in 49% of the vaginitis samples, and isolated in only 24% of the non-vaginitis samples. *Streptococcus* spp. were isolated in 29% of the vaginitis samples, and isolated in 11% of the non-vaginitis samples.

## Anaerobic Bacteria Isolation Results

Anaerobic bacteria were isolated in 24 (44%) of the vaginitis samples, and isolated in 11 (20%) of the non-vaginitis samples. The bacteria isolated include *Peptostreptococcus* sp., *Prevotella* sp., *Fusobacterium* sp., and *Clostridium perfringens* (Table 2). *Peptostreptococcus tetrans* was isolated in 31% of the vaginitis samples, and only in 9% of the non-vaginitis samples. The other anaerobic isolates did not show any distinct differences.

## Fungi Isolation Results

The fungal isolates include *Aspergillus* sp., *Penicillium* sp., and *Mucor* (Table 3).

**Table 1**. Aerobic bacteria isolated from bovine vaginas

| Aerobic Bacteria Isolated | Vaginitis (out of 55) | Percent seen in vaginitis | Non-vaginitis (out of 55) | Percent seen in non-vaginitis | Odds ratio |
|---|---|---|---|---|---|
| *Acinetobacter lwoffii* | 52 | 95% | 47 | 85% | 2.95 |
| *Actinobacillus ureae* | 1 | 2% | 0 | 0% | N/A* |
| *Arcanobacterium pyogenes* | 27 | 49% | 13 | 24% | 3.12 |
| *Arcanobacterium haemolyticum* | 17 | 31% | 15 | 27% | 1.19 |
| *Bacillus* spp. | 11 | 20% | 8 | 15% | 1.47 |
| *Chryseobacterium indologenes* | 6 | 11% | 0 | 0% | N/A* |
| *Citrobacter analonaticus* | 0 | 0% | 1 | 2% | 0 |
| *Comamonas acidovorans* | 3 | 5% | 0 | 0% | N/A* |
| *Corynebacterium aquaticum* | 0 | 0% | 1 | 2% | 0 |
| *Corynebacterium glucuronolyticum* | 16 | 29% | 5 | 9% | 4.1 |
| *Corynebacterium jeikeium* | 2 | 4% | 3 | 5% | 0.65 |
| *Corynebacterium renale* | 4 | 7% | 0 | 0% | N/A* |
| *Corynebacterium striatum* | 6 | 11% | 7 | 13% | 0.84 |
| *Corynebacterium* spp. | 13 | 24% | 11 | 20% | 1.24 |
| *Eikenella corrodens* | 2 | 4% | 0 | 0% | N/A* |
| *Erysipelothrix rhusiopathiae* | 1 | 2% | 0 | 0% | N/A* |
| *Escherichia. coli* | 18 | 33% | 12 | 22% | 1.74 |
| *Gemella morbillorum* | 17 | 31% | 12 | 22% | 1.6 |
| *Morganella morganii* | 19 | 35% | 15 | 27% | 1.41 |
| *Nocardia asteroides* | 0 | 0% | 1 | 2% | 0 |
| *Pasteurella haemolytica* | 3 | 5% | 0 | 0% | N/A* |
| *Pasteurella multocida* | 2 | 4% | 1 | 2% | 2.04 |
| *Pseudomonas aeruginosa* | 9 | 16% | 2 | 4% | 5.18 |
| *Serratia liquefaciens* | 2 | 4% | 1 | 2% | 2.04 |
| *Sphingomonas paucimobilis* | 1 | 2% | 0 | 0% | N/A* |
| *Staphylococcus simulans* | 0 | 0% | 1 | 2% | 0 |
| *Staphylococcus* spp. | 11 | 20% | 10 | 18% | 1.13 |
| *Stenotrophomonas maltophilia* | 6 | 11% | 1 | 2% | 6.86 |
| *Streptococcus acidominimus* | 8 | 15% | 2 | 4% | 4.51 |
| *Streptococcus agalactiae* | 5 | 9% | 2 | 4% | 2.65 |
| *Streptococcus bovis* | 2 | 4% | 0 | 0% | N/A* |
| *Streptococcus intermedius* | 11 | 20% | 4 | 7% | 3.44 |
| *Streptococcus uberis* | 2 | 4% | 1 | 2% | 2.04 |

* Odds ratio could not be calculated

**Table 2**. Anaerobic bacteria isolated from bovine vaginas

| Anaerobic Bacteria Isolated | Vaginitis (out of 55) | Percent seen in vaginitis | Non Vaginitis (out of 55) | Percent seen in non-vaginitis | Odds ratio |
|---|---|---|---|---|---|
| *Clostridium perfringens* | 0 | 0% | 1 | 2% | 0 |
| *Fusobacterium mortiferum* | 4 | 7% | 1 | 2% | 4.23 |
| *Fusobacterium varium* | 6 | 11% | 2 | 4% | 3.24 |
| *Peptostreptococcus anaerobius* | 7 | 13% | 3 | 5% | 2.53 |
| *Peptostreptococcus indolicus* | 0 | 0% | 1 | 2% | 0 |
| *Peptostreptococcus micros* | 7 | 13% | 1 | 2% | 7.00 |
| *Peptostreptococcus prevotii* | 2 | 4% | 0 | 0% | N/A* |
| *Peptostreptococcus tetrans* | 17 | 31% | 5 | 9% | 4.47 |
| *Prevotella loescheii* | 2 | 4% | 2 | 4% | 0 |
| *Prevotella oralis* | 3 | 5% | 2 | 4% | 1.02 |
| *Prevotella oris* | 1 | 2% | 1 | 2% | 0 |

* Odds ratio could not be calculated

**Table 3**. Fungi isolated from bovine vaginas

| Fungal Isolates | Vaginitis (out of 55) | Percent seen in vaginitis | Non-vaginitis (out of 55) | Percent seen in non-vaginitis | Odds ratio |
|---|---|---|---|---|---|
| *Aspergillus* sp. | 12 | 22% | 14 | 25% | .817 |
| *Mucor* sp. | 1 | 2% | 1 | 2% | 0 |
| *Penicillium* sp. | 24 | 44% | 15 | 27% | 2.07 |

Thirty (55%) of the vaginitis samples contained fugal isolates, while twenty-five (46%) of the non-vaginitis samples contained fugal isolates. Some samples contained both *Aspergillus* spp. and *Penicillium* spp. *Penicillium* spp. was isolated in 44% of the vaginitis samples, and was isolated in 27% of the non-vaginitis samples. *Aspergillus* spp. was isolated in 22% of the vaginitis samples, and was isolated in 25% of the non-vaginitis samples. One *Mucor* sp. was isolated in a vaginitis sample, and was also in only one of the non-vaginitis samples.

**Microaerophilic Bacteria Isolation Results**

There were no true microaerophilic bacteria isolated in any of the 110 vaginal samples.

***Campylobacter* sp. Isolation Results**

One vaginitis sample contained a possible *Campylobacter* sp. There was only one suspect colony that demonstrated colony morphology similar to that of *Campylobacter* sp. Several attempts to sub-culture the organism for identification were unsuccessful.

***Mycoplasma, Ureaplasma* and *Tritrichomonas foetus* Isolation Results**

Neither *Mycoplasma* sp. nor *Ureaplasma* sp. bacteria were isolated in any of the110 vagina samples taken. *Tritrichomonas foetus* was also not observed in any of the samples taken.

**Histopathologic Results**

Thirty-five of the 55 vaginitis samples demonstrated infection according to the histopathologic examination. The vaginitis samples showed neutrophil activity, lymphocytic infiltrates, intraepithelial lymphocytes, lymphoid follicles, and the presence

of macrophages. Twenty-one of the confirmed vaginitis samples had neutrophil activity in the vaginal fornix section, and only three had neutrophil activity in the uterine section. Six had neutrophil activity in both cross sections. Three of the 35 confirmed vaginitis samples were in early pregnancies. Two of the grossly identified vaginitis samples were autolyzed, and could not be confirmed by histopathologic examination.

Ten of the 55 non-vaginitis samples demonstrated signs of infection according to the histopathologic examination. Forty-five of the non-vaginitis did not show significant signs of infection. One of the non-vaginitis samples was in early pregnancy, and did not show signs of infection. Forty-three of the 110 samples showed evidence of mucoid hyperplasia, which occurred in both vaginitis and non-vaginitis samples.

# CHAPTER IV

## SUMMARY

Bacteria were isolated in all 110 bovine vaginas. The high proportion of aerobic bacteria isolated in both vaginitis and non-vaginitis was expected, with both yielding the same bacterial species. In past studies the types of bacteria isolated from normal animals and animals with vaginitis were virtually the same, with the only difference being vaginitis samples seemed to have a higher bacterial count.[2,6,8] The isolation of *Arcanobacterium pyogenes* in (49%) of the vaginitis samples demonstrates its clinical significance in reproductive disorders as reported in past studies.[3,5,11,12,13,15] The occurrence of *Acinetobacter lwoffii* in such high numbers in both vaginitis and non-vaginitis samples was not expected, but the bacteria has not been considered to be pathogenic. *Acinetobacter lwoffii* has been shown to occur in vaginitis as well as non-vaginitis samples.[2,4]

The absence of *Ureaplasma* and *Mycoplasma* was unexpected. Other studies have linked the symptom of bovine purulent vaginal discharge directly to the involvement of *Ureaplasma diversum.*[1] The majority of the studies done involving *Ureaplasma* sp. and *Mycoplasma* sp. focus on dairy cattle instead of beef cattle which was the focus of this study. Another explanation for the lack of *Ureaplasma* sp. and *Mycoplasma* sp. is that the bacteria may have been present in the specimens, but may have been in such low numbers that they were not able to be cultured. Since specimens were cultured with the correct media and incubation enviroment, we are confident that the organisms were not there.

There is limited published research involving fungal isolates in reproductive infections in animals. *Aspergillus* sp., and *Mucor* sp. can cause sporadic abortion in cows, but in most cases were considered to be of doubtful significance.[11] There was not a direct correlation between vaginitis and non-vaginitis fungal isolations. Both vaginitis and non-vaginitis samples contained the same fungal isolates in relatively close numbers. The only exception being that *Penicillium* sp. was isolated in 44% of the vaginitis samples, and only in 27% of the non-vaginitis samples.

The absence of *Tritrichomonas foetus* was expected. *Tritrichomonas foetus* is one of the leading causes of infectious infertility.[15] The organism is usually isolated from the smegma of bulls, pyometra fluid from cows, or abomasal contents of an aborted fetus.[11]

As other studies have concluded, the organisms isolated in vaginitis and non-vaginitis samples are similar.[8,14] The noticeable difference between the two groups is the occurrence of particular bacterial isolates in higher numbers. *Escherichia coli*, *Streptococcus* spp., *Corynebacterium* spp., and *Staphylococcus* spp. are causes of nonspecific infections, while *Arcanobacterium pyogenes*, *Campylobacter* sp., *Ureaplasma* sp., *Mycoplasma* sp., and *Tritrichomonas foetus* are specific infections.[13]

Both histopathologic findings and bacteriologic findings provide more reliable information than just clinical examination alone. Ten of the samples that grossly appeared to be clinically normal non-vaginitis demonstrated evidence of infection according to the histopathologic examination. Twenty of the grossly diagnosed vaginitis samples did not show signs of infection according to the histopathologic examination.

This may suggest that there may be other agents besides bacteria that bring about these infections.

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